Role of ATM kinase in ionizing radiation induced DNA damage response in human neural stem/progenitor cells and differentiated cell types

Jarah A. Meador[1], Domenico Delia[2] and Adayabalam S. Balajee[1]

[1]Center for Radiological Research, Department of Radiation Oncology, Columbia University Medical Center, 630 West, 168th Street, New York, NY, USA. [2] Department of Experimental Oncology, Fondazione IRCSS Istituto Nazionale Tumori, Milan, Italy.

Tissue microenvironment contains a heterogeneous mixture of stem/progenitor cells (undifferentiated and pluripotent) and differentiated cells. Our current knowledge of how these different cell types respond to low dose radiation is highly restricted. Further, it is not clear whether DNA repair mechanisms differ between replicative multipotent stem cells and non-replicative post-mitotic cells such as neurons. Therefore, knowledge of DNA damage response (DDR) mechanisms for different cell types in a tissue microenvironment is essential for assessing ionizing radiation (IR) induced human health risks. Frequent occurrence of neurodegenerative features associated with human radiosensitive patients such as ataxia telangiectasia and Nijmegen breakage syndrome highlight the importance of DNA double strand break (DSB) repair pathway in maintaining the functional integrity of the central nervous system. Although, the importance of ATM, ATR and DNA-PK has been well established in cell cycle checkpoint and DNA repair activities in proliferating cell systems, their precise role in replicatively quiescent post-mitotic neurons is essentially unknown. In this study, we have analyzed the role of ATM in ionizing radiation (IR) induced DNA damage response both in primary (ENStem-A) and immortalized (ihNSC and ReNcell VM) neural stem/progenitor cells and their differentiated cell lineages. These cell systems retain self-renewal and multi-lineage differentiation capabilities even after extended passages in culture. These features may enable us to detect the mechanistic differences in DNA repair activities between multipotent neural stem cells and differentiated neural cell types (neurons, oligodendrocytes and astrocytes). To directly determine the role of ATM, ReNVM cells with stably suppressed ATM expression were generated by transduction of lentiviral shRNA vectors targeting the nucleotide sequences 268-286 and 1267-1285 of the ATM transcript. Initially, we evaluated the effect of ATM kinase on neurogenesis. Inhibition of either ATM or ATM/ATR kinases greatly affected the neuronal maturation as judged by the neurite growth. Further, ATM suppression impaired the differentiation of neural stem/progenitor cells into glial cell lineages. In sharp contrast to the slow growth of ATM deficient primary fibroblasts, ATM suppressed neural progenitor cells exhibited a slightly increased proliferation potential in culture. Most strikingly, ATM ablation also attenuated the differentiation and oxidative DNA damage induced apoptotic death of neurons and glial cells thereby increasing the accumulation of genomic instability features.

Induction of DNA damage and repair was next assessed in neural stem/progenitor cells and neurons following exposure to varying doses (0.1Gy, 0.25Gy. 0.5Gy, 1Gy and 2.5Gy) of γ-rays radiation.

The initial induction of 53BP1 and γ-H2AX foci reflective of DNA double strand breaks was essentially similar between ATM proficient neural stem/progenitor cells and differentiated neurons at all of the radiation doses examined. However, cells with persistent 53BP1 and γ-H2AX foci analyzed 24 hr and 48 hr after IR exposure were consistently higher in neurons than in neural stem/progenitor cells. Moreover, ATM deficiency further enhanced the proportion of neurons with persistent 53BP1 and γ-H2AX foci. Consistent with this observation, micronuclei formation was also increased in neurons as compared to neural stem/progenitor cells. Collectively, these results suggest that the DSB repair efficiency may be reduced in neurons. We next wished to determine whether alterations in the expression of DNA repair genes affect the fidelity and efficiency of DNA repair mechanisms in the newly differentiated MAP2 positive neurons. For this purpose, comparative analysis of gene expression was performed in multipotent adult stem cells and differentiated neurons using a quantitative real time PCR profiler array (SA Biosciences, Frederick, MD, USA) for a total of 84 genes that participate in diverse DNA damage signaling/repair pathways (Nucleotide Excision Repair, Base Excision Repair, Mismatch Repair, Non-homologous End Joining Repair and Homologous Recombination Repair). Of interest, we found that ATM and DNA-PK showed a biphasic pattern with an initial decline in expression in neurons followed by an elevated expression in oligodendrocytes and astrocytes. In contrast, ATR kinase expression was drastically reduced both at mRNA and protein levels in differentiated neural cell types (neurons and glial cells) in comparison to neural stem/progenitor cells. Thus, it appears that ATR, an essential factor for S-phase checkpoint and DNA repair in proliferating cells, is not required for differentiation associated repair that is operative in post-mitotic neurons. In addition to ATM, ATR and DNA-PK, many important proteins involved in DNA repair and cell cycle checkpoint regulation also showed reduced expression in neurons. Some of these include BRCA1, Chk1, Chk2, ERCC2, FANCG, Exo1, MLH1, MLH3, MSH2, MSH3, OGG1, PCNA, PMS1, Rad18, Rad21, Rad50, Rad51, Rev1, RPA1, P53, p73, UNG, XPA, XPC, XRCC1, XRCC2 and XRCC3. Interestingly, expression levels of 7 genes (BTG2, ERCC1, GML, GTF2H2, MAPK12, Rad1 and SUMO1) were found to be specifically higher in neurons as compared to multipotent stem cells. These results suggest that down regulation of DNA repair genes may be responsible for the differential DNA damage response of neurons to IR exposure. As efficient ATM activation depends on MRN complex, we are assessing the role of Mre11 and Nbs1 gene products in adult neurogenesis and neuroprotection after exposure to low doses of low LET radiation. Our findings highlight the potential of ENStem-A and ReNcell VM as ideal *in vitro* model systems to thoroughly assess the role of important DNA repair proteins in neurogenesis and /or neurodegeneration.

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